Enhanced Sensitivity for Biomarker Characterization in Petroleum Using Triple Quadrupole GC/MS and Backflushing

Application Note

Environmental

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Abstract

A rapid, reliable method for the routine detection and quantification of biomarkers in petroleum was developed using the Agilent 7890A/7000A Series Triple Quadrupole GC/MS with backflushing using a Pressure Controlled Tee configuration. In a single run, diverse biomarkers from several transitions can be detected, confirmed, and quantified at levels as low as 2 ppm, with RSDs well below 5%. This method is suitable for "fingerprinting" of petroleum samples and the deconvolution of oil mixtures in complex, multisource petroleum systems.

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Introduction

Petroleum biomarkers are complex molecular fossils derived from once living organisms [1]. These compounds provide unique clues to the identity of source rocks from which petroleum samples are derived. This information includes the biological source organisms which generated the organic matter, the environmental conditions that prevailed in the water column and sediment at the time, the thermal history of both the rock and the oil, and the degree of microbial biodegradation. Biomarkers are used in conjunction with other geochemical parameters to help solve oil exploration, development, and production problems. They provide much more detailed information about petroleum source and history than nonbiomarker analysis (bulk isotopes, elemental analysis, and so forth) alone.

High resolution mass spectrometry (HRMS) is often used to analyze biomarkers in petroleum, due to its ability to provide quantitative data for compounds present in complex mixtures. However, HRMS requires a significant financial investment as well as highly trained operators to assure valid results. Triple Quadrupole GC/MS offers a viable alternative for the rapid, routine analysis of biomarkers in petroleum, providing excellent precision, sensitivity, selectivity, and dynamic range. Implementing GC backflushing in the acquisition method improves data quality robustness, due to the very complex and varied nature of petroleum samples.

Experimental

Standards and Samples

STANFORD-1 is a new external standard for quantitative biomarker analysis. It is a mixture of pure biomarker standards and paraffin-free saturate fractions from Paleozoic, Mesozoic, Cenozoic, biodegraded, terrestrially-influenced, carbonate/evaporate-sourced, and open-marine sourced petroleum samples. It contains known quantities of most, if not all, commonly used biomarkers and two internal standards, BTI-6 and 5-β cholane, which are useful for quantifying hopane and sterane biomarkers across diverse GC/MS systems.

C30 sterane fractions were prepared using standard normal phase liquid chromatography techniques, n-alkane removal, and proprietary molecular sieve and HPLC techniques for the final enrichment of target compounds. Two compounds which coelute with n-propylcholestane (4-methylstigmastane and hopane) were completely removed from the sample to avoid known interference with the *m/z* 414→217 transition.

Instruments

The experiments were performed on an Agilent 7890A gas chromatograph equipped with a split/splitless inlet, an Agilent 7000A Triple Quadrupole GC/MS with Triple-Axis Detector, and an Agilent 7683B automatic liquid sampler (ALS). The split/splitless inlet is fitted with a deactivated, helical double taper injection liner (p/n 5188-5398). Injections were made using a 10-µL syringe (p/n 9301-0713). A variety of configurations was explored to examine possible improvements in analysis time. Ultimately, two configurations were used for the experiments, and the instrument conditions and specific configurations are listed in Table 1.

MS SRM Parameters

The MS/MS parameters used in the analysis of the petroleum samples are shown in Tables 2 and 3 and in the Figure 6 legend. Experience with HRMS metastable transitions was used to select these precursor and product ions, and an extensive study of product ions was not performed.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC Run Conditions	60 m Configuration	40 m Configuration
Column	Two 30 m x 0.25 mm x 0.25 µm DB-1MS Ultra Inert columns (p/n123-0132UI)	Two 20 m x 0.18 mm x 0.18 µm DB-1MS Ultra Inert columns (p/n 121-0122UI)
Inlet temperature	325 °C	325 °C
Inlet pressure	19.197 psi	17.13 psi
Carrier gas	Helium, constant flow mode	Hydrogen, constant flow mode
Flow rate	Column 1: 1.15 mL/min; Column 2: 1.20 mL/min	Column 1: 0.95 mL/min; Column 2: 1.0 mL/min
Injection mode	Pulsed splitless (50 psi until 1 min)	Pulsed splitless (50 psi until 0.75 min)
Oven program	50 °C (1 min hold), then 40 °C/min to 140 °C for 0 min, then 2 °C/min to 313.5 °C for 0 min	40 °C (0.6 min hold), then 40 °C/min to 140 °C for 0.5 min, then 3.4 °C/min to 300 °C for 1 min
Column velocity	Column 1: 27.636; Column 2: 39.923 cm/s	Column 1: 45.449; Column 2: 65.944 cm/s
Injection volume	1 µL	1 µL
Transfer line temperature	325 °C	325 °C
GC Post-Run Conditions		
Backflush device	Purged Ultimate Union (p/n G3186-60580) controlled by a Electronic Pneumatic Control (EPC) (p/n G3470A)	Purged Ultimate Union (p/n G3186-60580) controlled by a Electronic Pneumatic Control (EPC) (p/n G3471A)
Backflush conditions	–4 mL/min at 325 °C for 7 min	–4 mL/min at 325 °C for 5 min
MS Conditions		
Tune	Autotune	Autotune
Delta EMV	70 eV	70 eV
Acquisition parameters	EI; selected reaction monitoring	EI; selected reaction monitoring
Solvent delay	5 min	3 min
MS temperatures	Source 250 °C; Quadrupoles 150 °C	Source 250°C; Quadrupoles 150 °C

Table 2. Analysis Parameters for Precision Experiments*

Compound	Transition (*m/z*)
Stigmastane	400.4 → 217.2
Homohopane (22S)	426.4 → 191.2
n-propylcholestane	414.4 → 217.2
27-nordiacholestane (13β,17α(H),20S)	358.4 → 217.2
27-norcholestane	358.4 → 217.2
4-methylstigmastane	414.4 → 231.2
Dinosterane	414.4 → 98.1
Hopane	412.4 → 369.4
5β-Cholane (ISTD)	330.3 → 217.2

*The method contained 17 transitions in total. The dwell time and collision energy used for each transition was 50 msec and 5 eV, respectively, using the 60 m configuration.

Results and Discussion

Backflushing with a Pressure Controlled Tee Configuration

Backflushing was used to remove higher boiling substances from the column prior to each subsequent run. Using this technique, late eluting peaks are flushed out of the inlet split flow vent instead of driving them through the entire length of column and into the mass spectrometer. Backflushing reduces accumulated chemical noise due to carryover (which can be observed even in SRM mode as a rising baseline) and the cycle time of the analysis, thus increasing throughput. System uptime is also increased, due to reduced maintenance of the columns and MS detector. The suite of Agilent Capillary

Flow Technology modules comprises a proprietary solution that enables easy and rapid backflushing with minimal dead volumes for maintaining chromatographic resolution. It also uses ferrules and fittings that eliminate leaks. All Capillary Flow Technology modules require the use of an Auxiliary Electronic Pneumatic Control (EPC) module or a Pneumatic Control Module (PCM) to provide a precisely-controlled second source of gas that directs the column flow to the appropriate column or detector. During analysis, the EPC module supplies a pressure slightly above the pressure of the carrier gas through the column. When backflushing, the inlet pressure is dropped and the EPC module pressure is increased, forcing the flow to reverse through the column and out the split vent.

A quick and simple approach to backflushing is to use a Capillary Flow Technology device in the middle of the analytical column [2–4]. As an example employed here, instead of using a 40-m column, two 20-m columns are used and connected by an ultralow dead volume Purged Ultimate Union in a Pressure Controlled Tee (PCT) configuration (Figure 1). The EPC module adds just enough makeup gas to match that from the first column, so there is very little flow addition and subsequent decrease in sensitivity due to suboptimal carrier gas flows into the mass spectrometer. As a general rule, the flow for column 2 is set to be 0.02 to 0.05 mL/min greater than that for column 1. Backflushing in this configuration is accomplished simply by reducing the flow or pressure in the first column and increasing it in the second column.

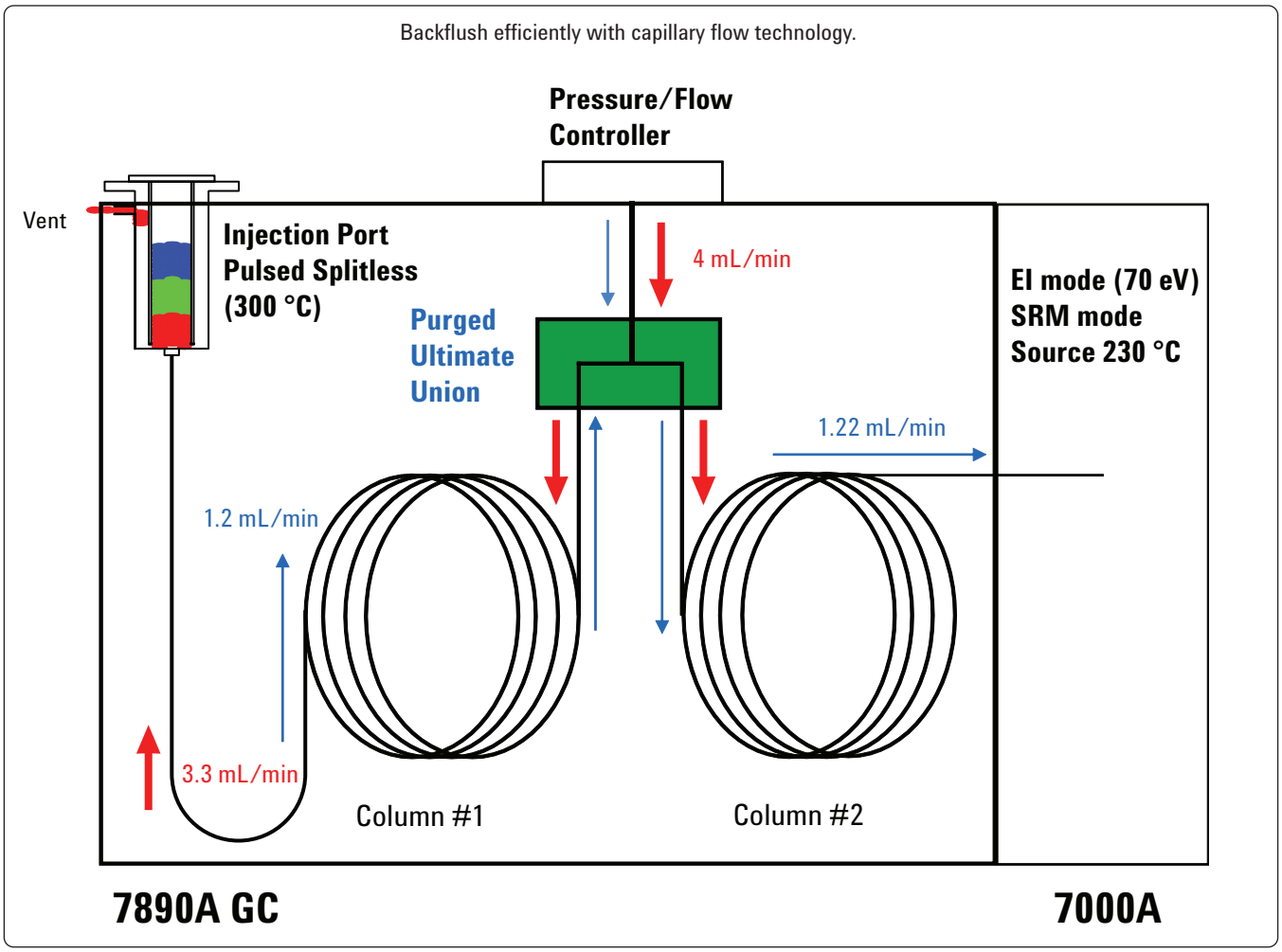

Figure 1. Schematic of the Pressure Controlled Tee GC/MS configuration. The narrower (blue) lines indicate the forward flow during analysis and the thicker (red) lines indicate the backflushing post-run state.

Figure 2 illustrates the advantages of backflushing with the PCT configuration. Typical hydrocarbon GC/MS analysis requires long cycle times due to long hold times at high oven temperatures to avoid contaminating subsequent analyses with carryover of high-boiling components (top chromatogram). Using backflush, targeted volatile components, in this case those eluting within 25 minutes, can be analyzed with significantly shorter cycle times, eliminating the need for column baking and extended GC run times (bottom chromatogram). High boiling hydrocarbons are not retained and column degradation by "permanently" absorbed components and high temperature hold times is decreased. In the example shown, cycle times are reduced from over 100 minutes to less than 30 minutes, and a blank injection after backflushing reveals no high-boiling components and only the baseline rise associated with column bleed.

Faster Analysis of Biomarkers

Run times can be accelerated 30 minutes per cycle without loss in chromatographic resolution or substantial loss in signal by switching from a 60-m (0.25-mm id) column with helium carrier gas to a 40-m (0.18-mm id) column with hydrogen carrier gas (Figure 3). The speed of the 7000A Triple Quadrupole mass spectrometer in SRM mode required only a change in dwell time from 50 to 20 msec to record the required 17 transitions with the same number of scans over the peaks. Because the 7000A Triple Quadrupole MS allows dwell times as short as 1 msec, even faster analysis is possible. An experimental comparison with an uninterrupted 60-m column (results not shown) demonstrated that the insertion of the PCT configuration results in no degradation in chromatography due to the low dead-volume of the Purged Ultimate Union.

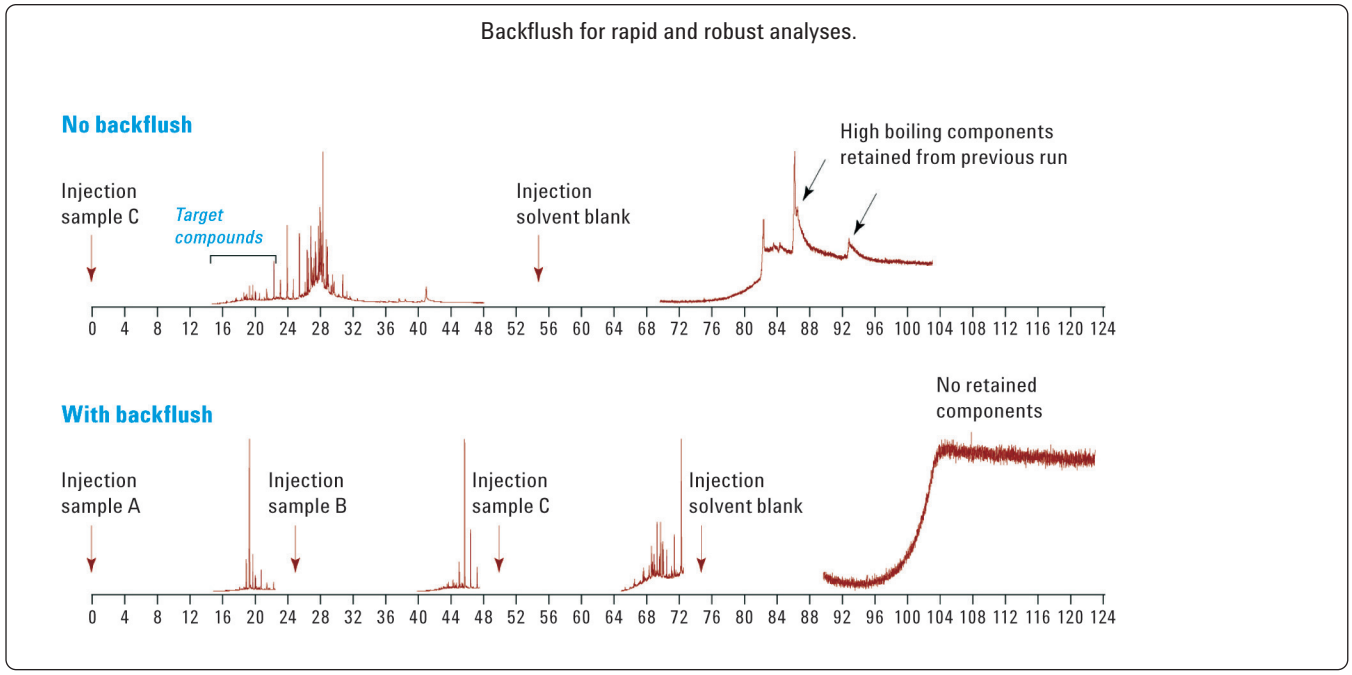

Figure 2. Petroleum samples, including one from Williston Basin source rocks (Sample C) which contains many late eluting, high molecular weight hydrocarbons, were analyzed without (top) and with (bottom) backflushing (40 m configuration). The target compounds comprise a subset of the total number of possible compounds in any injected sample and are indicated by brackets in the top chromatogram. As in a typical analysis, a sequence of samples was analyzed from three sources using the backflushing method in the bottom trace, followed by a solvent blank injection which demonstrated the lack of retained components.

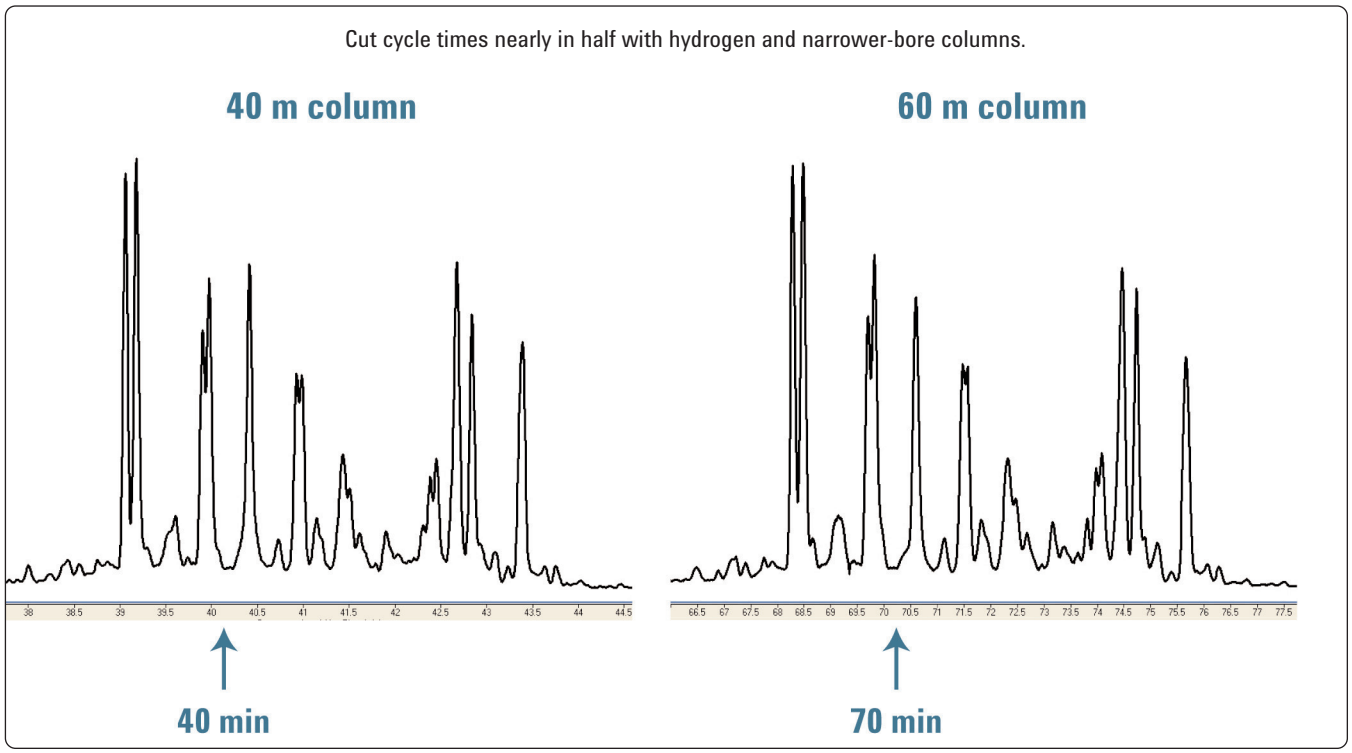

Figure 3. C28 steranes were analyzed using m/z transition 386→217 on either a 60 meter, 0.25 µm column and helium carrier gas, or a 40 meter, 0.18 µm column with hydrogen carrier gas. Employing hydrogen and the smaller bore column reduces analytical time significantly without loss in compound resolution.

Sensitivity, Selectivity and Precision

Routine biomarker analysis in petroleum samples requires precise determination of the abundance of a large number of individual compounds which can vary over a large range of concentrations in these complex mixtures. This precision allows the distinction of differences between petroleum samples with subtly different source or post-generation history. Results for ten sequential runs of the STANFORD-1 standard demonstrate that calculated concentrations of eight different compounds using several different transitions with widely varying concentrations is quite precise (Table 2, Figure 4a).

Most relative standard deviations (RSDs) were well below 5%. The only compound that gave an RSD higher than 5% (dinosterane) was present at a very low concentration (~2 ppm) and required manual integration for quantification. In addition, the calculated concentrations of the compounds were within a few percent of the expected concentration across all ten runs, except for the manually integrated dinosterane (Figure 4b). This precision demonstrates the ability of the Triple Quadrupole GC/MS system to distinguish subtle variations in petroleum composition for traditional biomarker studies, reservoir partitioning studies, and three-dimensional basin modeling.

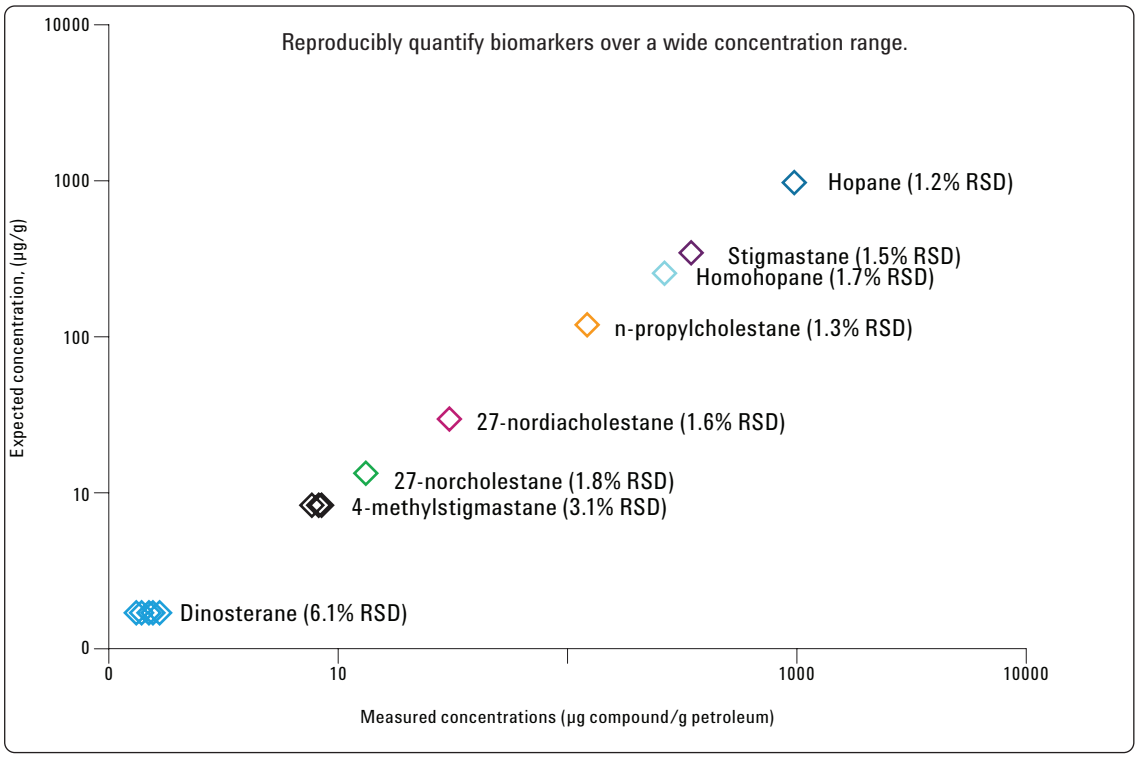

Figure 4a. Precision experiment results for eight biomarkers of widely varying concentrations contained within the STANFORD-1 standard. Ten sequential analyses were performed over a 15 hour period using the 60-m column PCT configuration. See Table 3 for transitions.

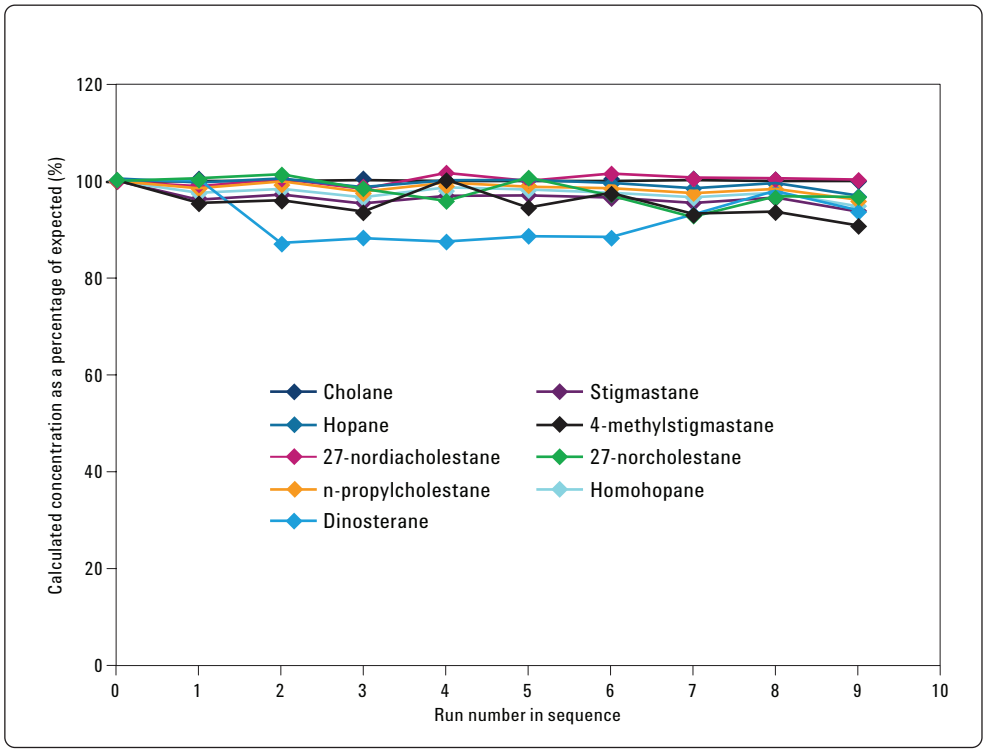

Figure 4b. The data from the analysis described in Figure 4a were plotted as calculated concentration of each biomarker versus the expected concentration over 10 analyses.

Deconvolving Oil Mixtures

A sophisticated understanding of petroleum systems requires the recognition and deconvolution of oil samples derived from more than one source rock. This problem is common where stacked source rocks exist in sedimentary basins (Figure 5). For this work a series of laboratory mixtures consisting of a marine petroleum endmember and a lacustrine endmember were analyzed for stigmastane, a ubiquitous component present in petroleum from both sources, and n-propylcholestane, a compound unique to oil from marine source rock.

As the ubiquitous component must be measured on a different SRM transition and is an order of magnitude more abundant in the marine oil, transition ratio stability and a large instrumental dynamic range are necessary to accurately identify small marine petroleum inputs in lacustrine source rock samples. The data demonstrate that mixtures as low as 0.2% (v/v) in the minor marine component can be accurately determined (Figure 6).

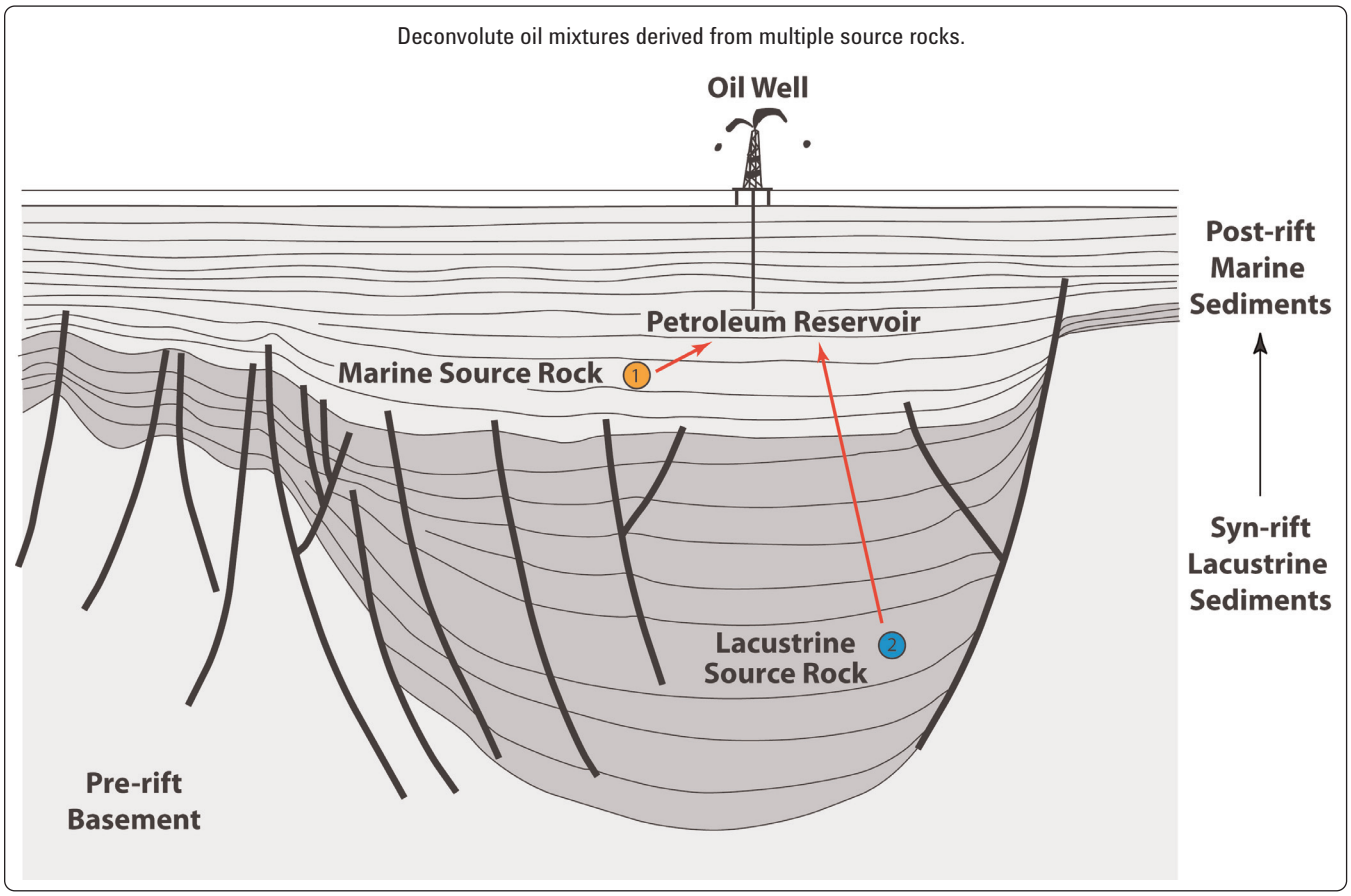

Figure 5. Diagram of an oil deposit containing source rocks from both marine (1) and lacustrine (2) sources.

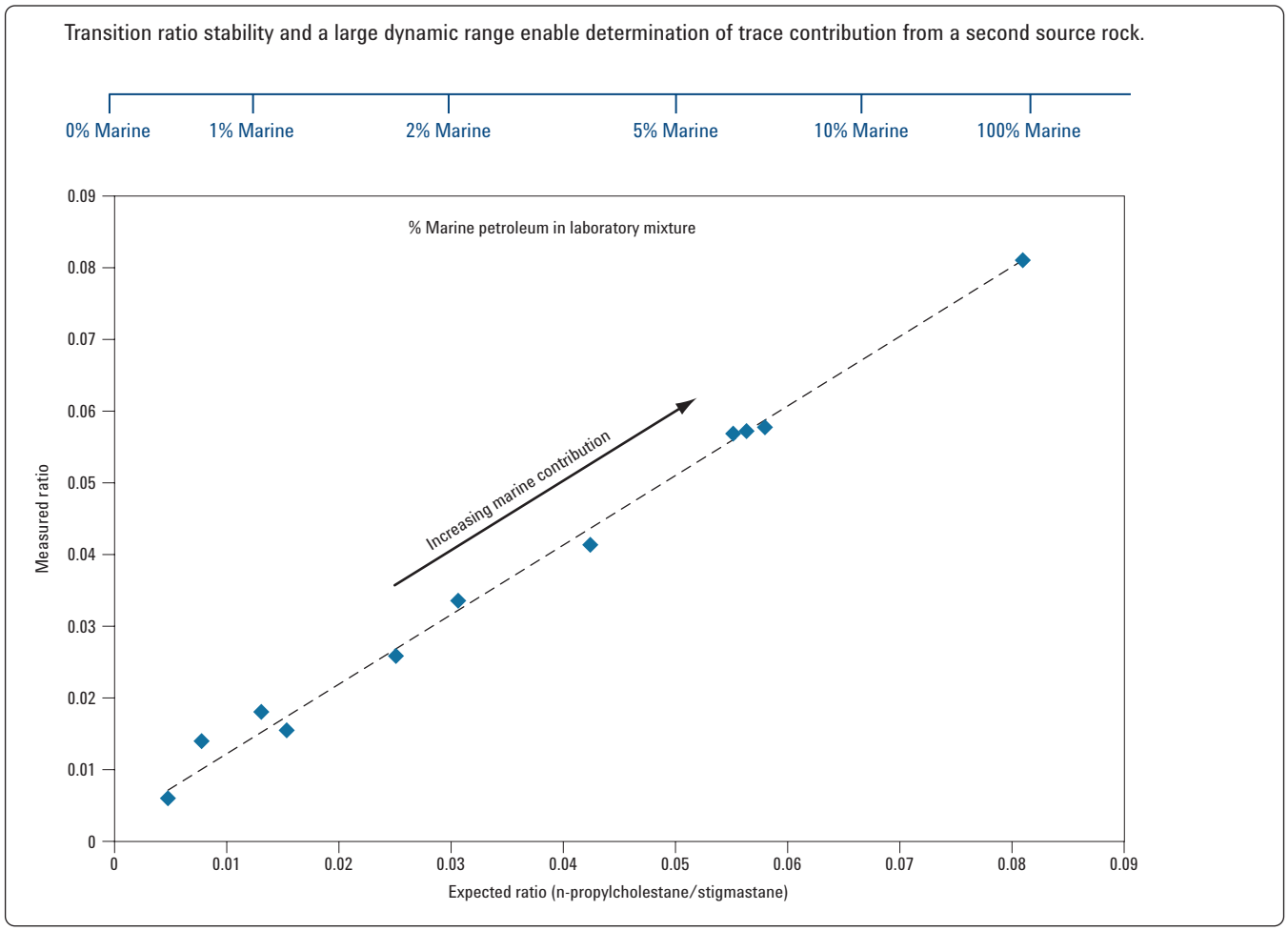

Figure 6. A series of laboratory mixtures consisting of various percentages of a marine petroleum sample in a lacustrine sample were analyzed for stigmastane, a ubiquitous component present in petroleum from both sources, and n-propylcholestane, a compound unique to oil from lacustrine source rock. The measured ratio of the two compounds was then plotted versus the expected ratio. Transitions monitored were: n-propylcholestane, m/z 414.4→217.2; stigmastane, m/z 400.4→217.2.

Conclusions

The Agilent 7000A Triple Quadrupole MS with 7890 GC using backflushing is a viable approach to the routine analysis of petroleum biomarkers, providing increased sensitivity, better selectivity and the potential to greatly reduce analysis time versus traditional GC/MS analysis. Column backflush provides higher sample throughput with lower carryover and source maintenance, and the use of hydrogen carrier gas and narrower bore columns reduces run times nearly two-fold at no significant loss in chromatographic resolution. The SRM speed, linearity, dynamic range and transition ratio stability of the 7000A Triple Quadrupole mass spectrometer enable quantitative characterization for the fingerprinting of petroleum samples and the deconvolution of complex petroleum mixtures.

References

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